Identification of Feasible Metabolic Fluxes and Metabolite Concentrations using Large-scale Kinetic Models

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Abstract

The constraints imposed during modeling must satisfy biologically representative phenotypes of the studied organism. Simultaneously, identification and analysis of these constraints enhances our understanding of the evolution/operational paradigms of the organism. It was postulated by Varma and Palsson[1] that it is possible to define limits on metabolic behavior using flux balance analysis, but in order to accurately capture the metabolic responses, detailed information about enzyme kinetics and their regulation is needed. Since development of mechanistic kinetic models is a difficult task due to uncertainty in kinetic properties of enzymes, a substantial number of recent works consider only the mass action (MA) term in their model formulation. As kinetics is one of crucial factors in governing the metabolic capabilities of a cell, i.e. realizable metabolic flux and concentration states, considering only the mass action term does not necessarily provide a realistic description of the feasible space of fluxes and concentrations. In this work, using the ORACLE[2] framework, we constructed a large-scale mechanistic kinetic model of optimally grown *E. coli* that considers the enzyme saturations as observed in biological systems. Using this model, we performed an analysis of the complex interplay between stoichiometry, thermodynamics, and kinetics in determining flexibility and capabilities of metabolic networks. Our analysis indicates that enzyme saturation is an important and necessary consideration in modeling metabolic networks. Extended ranges of feasibility, both in the space of metabolic fluxes and metabolite concentrations, of kinetic models involving the enzyme saturation suggests that the enzymes in metabolic networks have evolved to function at different saturation states so as to ensure higher flexibility and robustness of the cell.

ORACLE methodology

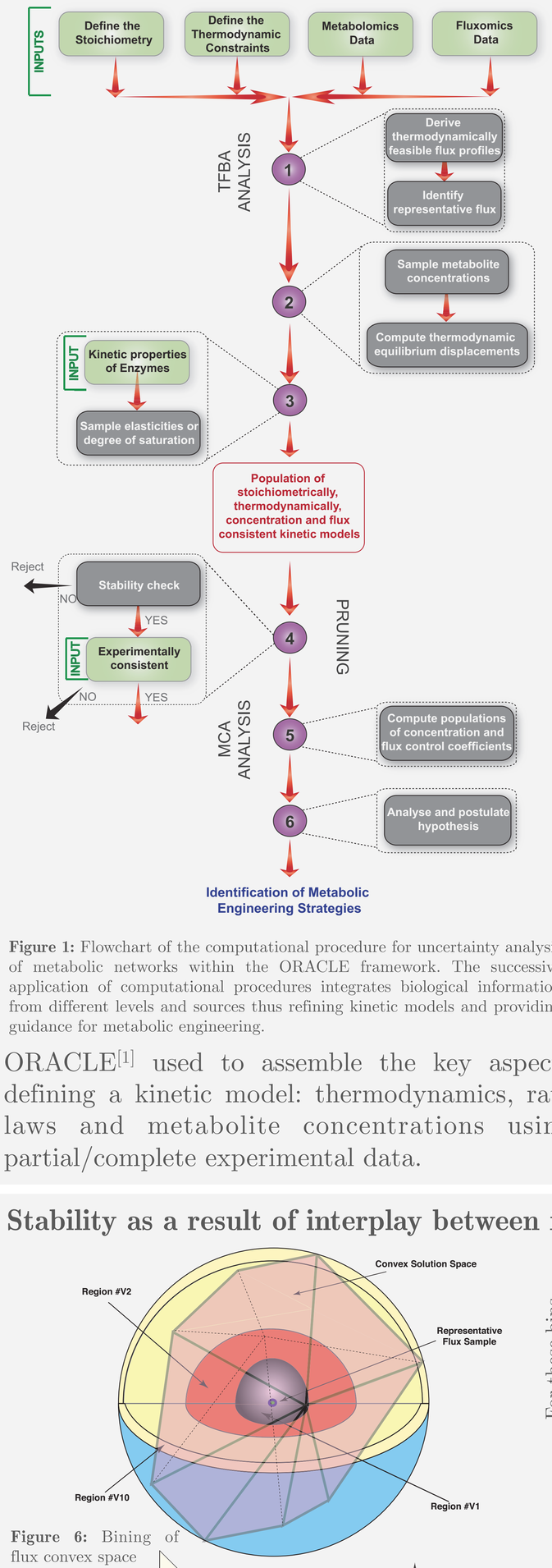

Figure 1: Flowchart of the computational procedure for uncertainty analysis of metabolic networks within the ORACLE framework. The successive application of computational procedures integrates biological information from different levels and sources thus refining kinetic models and providing guidance for metabolic engineering.

ORACLE[1] used to assemble the key aspects defining a kinetic model: thermodynamics, rate laws and metabolite concentrations using partial/complete experimental data.

Consistently reduced *E. coli* model

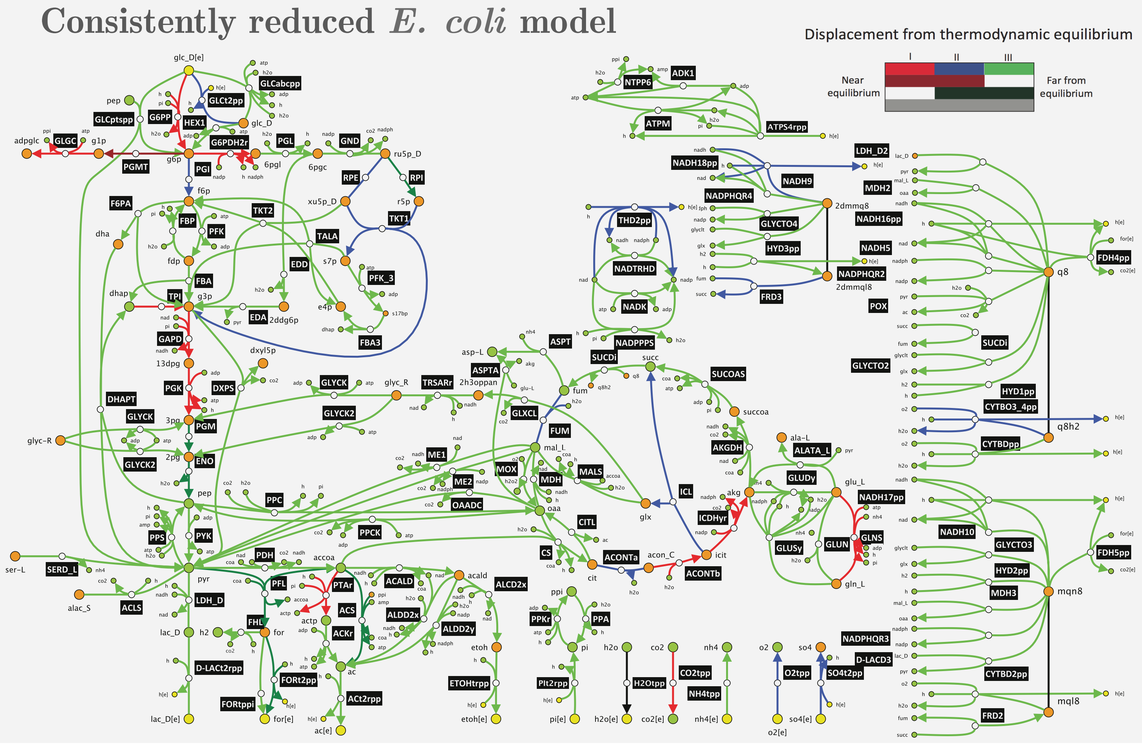

Figure 2 Thermodynamic displacements of the reactions in the consistently reduced *E. coli.* network. The network included 146 reactions and 90 metabolites, and it is fully balanced even with respect to the small molecules such as CO_2, NH_3, and PO_4, and with respect to protons and electrons.

Derived from a genome scale *E. coli* reconstruction, the reduced core *E. coli* model consisted of 146 reactions and 90 metabolites. We used fixed glucose uptake of 10 mmol/gdw-hr, maximum oxygen uptake of 20 mmol/gdw-hr. Using tFBA, we were able to characterize the equilibrium displacements of the reactions and study the equilibrium state of the network.

Stability landscape of the flux and concentration space

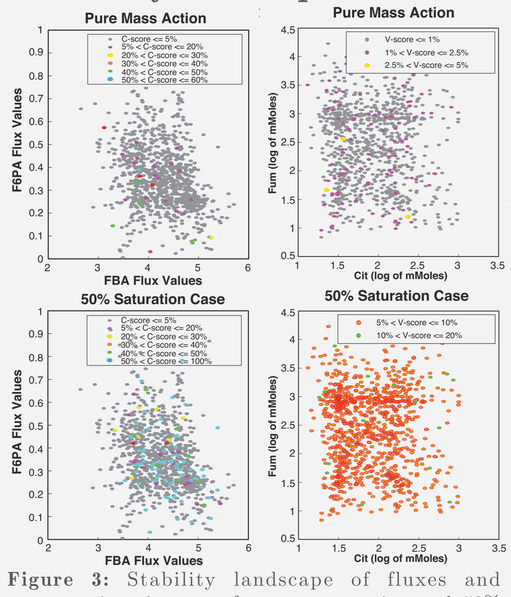

Figure 3: Stability landscape of fluxes and concentrations in case of pure mass action and 50% enzyme saturation case. Overall we see increased stability for the flux and concentration samples for enzyme kinetics with 50% saturation.

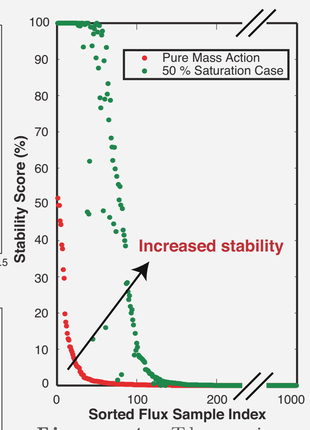

Figure 4: There is a consistent increase in stability for all flux samples for enzyme kinetics with 50% saturation.

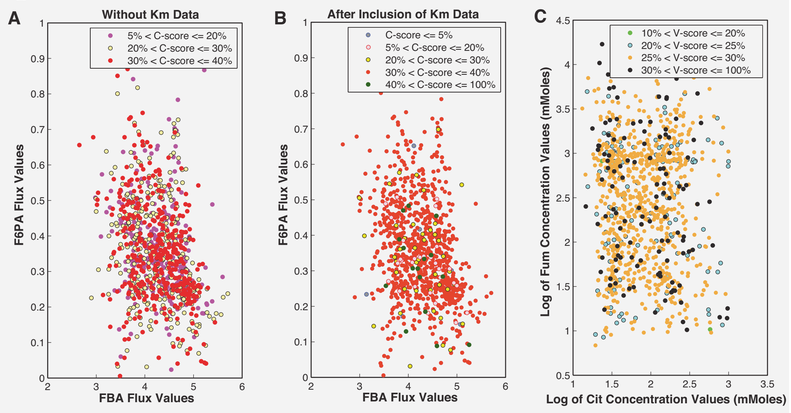

Figure 5: Stability landscape of flux and concentration samples in case of full range of enzyme saturation levels (0-1). (A) Cross-plot of flux samples of FBA versus F6PA depicting *mean* C-score calculated without inclusion of any experimental/database K_m measurements, (B) *Mean* C-score with experimental/database kinetic information of K_m measurements incorporated. (C) Cross plot of concentration samples for citrate versus fumarate.

We observed consistently higher stability scores for both the flux (*C-score*) and concentration (*V-score*) samples in the case of models with mechanistic enzyme kinetics.

Stability as a result of interplay between flux and concentration space

Figure 6: Bining of flux convex space

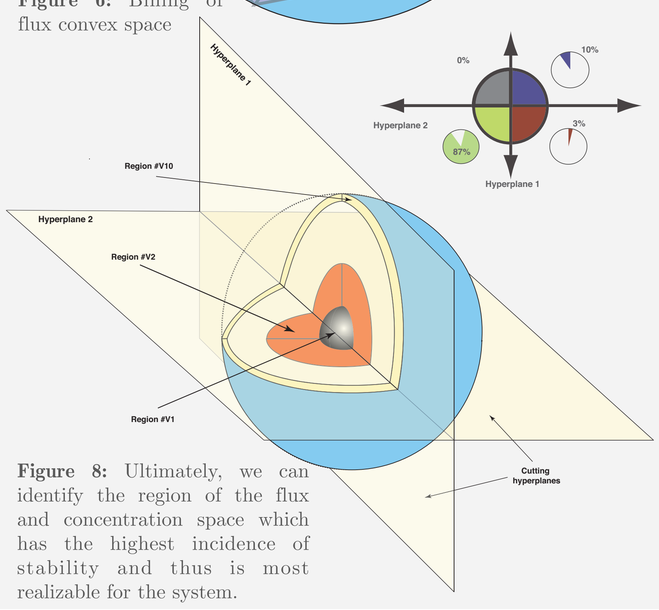

Figure 8: Ultimately, we can identify the region of the flux and concentration space which has the highest incidence of stability and thus is most realizable for the system.

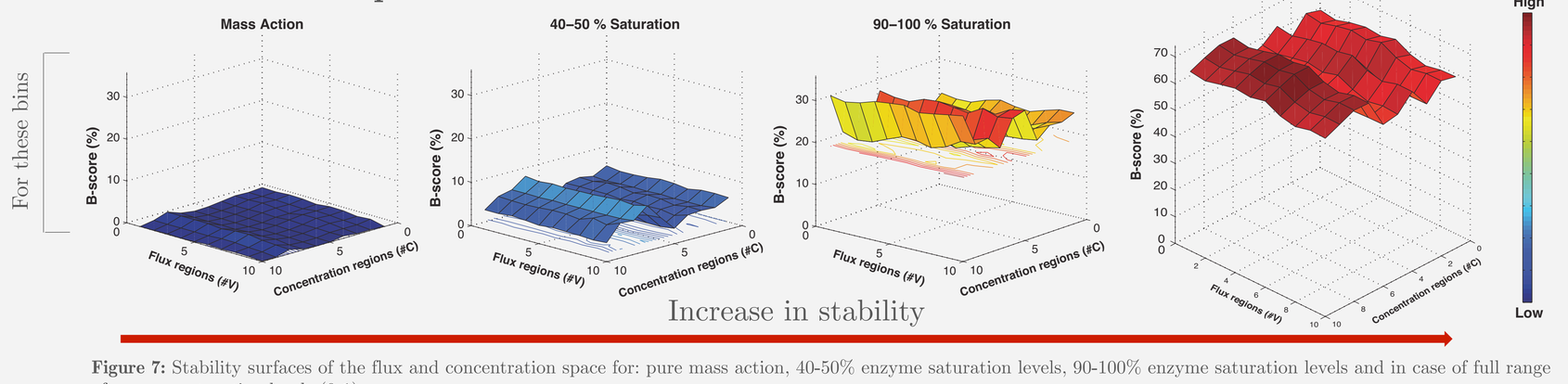

Figure 7: Stability surfaces of the flux and concentration space for: pure mass action, 40-50% enzyme saturation levels, 90-100% enzyme saturation levels and in case of full range of enzyme saturation levels (0-1).

To identify *iso-stability* regions in the high-dimensional flux and/or concentration space, we clustered the flux and the concentration samples into 100 bins and quantified the stability *B-score*. Analysis of the stability of flux-concentration bins indicated very limited variability of B-scores as a function of flux samples as compared to a function of concentration samples. Furthermore, these results suggest that the saturation kinetics of enzymes contribute to the stability of the system, and they allow a wider range of kinetically feasible concentration profiles.

Conclusion

- ✓ Assessing the feasibility of concentration and flux profiles using mass action kinetics, can lead to overly conservative assessments thus neglecting concentration and flux profiles that are likely to correspond to a physiological condition of the system
- ✓ The fact that enzyme saturation terms and inclusion of experimentally observed kinetic data consistently increased the stability of the kinetic models, i.e. their feasible space of fluxes and concentrations increased, indicates that enzymes have evolved in a way to increase the flexibility and thus the viability and adaptability of living organisms.

References

[1] Varma, A., and Palsson, B.O. (1993). Metabolic capabilities of Escherichia coli: I. synthesis of biosynthetic precursors and cofactors. J Theor Biol *165*, 477-502.

[2] Miskovic, L., and Hatzimanikatis, V. (2010). Production of biofuels and biochemicals: in need of an ORACLE. Trends in Biotechnology *28*, 391-397.